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STUDY OF SEQUENCE OF HISTOPATHOLOGICAL CHANGES IN HYPERGLYCAEMIA-INDUCED EXPERIMENTAL DIABETIC NEPHROPATHY IN WISTAR RAT MODEL

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Abstract

The sequence of histopathological changes in hyperglycaemia-induced nephrotoxicity as well as alteration in renal parameters is still not well established in diabetic models. Hyperglycaemic ambience has been shown to generate oxidative stress which becomes a trigger for further degenerative changes that occur in the microenvironment of the kidney. This study was therefore intended to investigate histopathological alterations in vascular, glomerular and tubulointerstitial compartments of the renal tissues, and the corresponding changes in values of oxidative stress markers, creatinine clearance, proteinuria and serum creatinine concentration in a duration of three, seven and twelve weeks of sustained hyperglycaemia in diabetic. The experiment included four groups of adult Wistar rat, Group A (Normal Control, treated with normal saline), Groups B, C and D were induced with diabetes (treated with 65mg/kg body weight of streptozotocin) and allowed for 3 weeks 7 weeks and 12 weeks respectively. At termination, Oxidative stress markers were analyzed using Oxidative stress marker kits. A 24 hours urine collection was obtained from metabolic cages few hours before sacrifice and used for renal analysis and histopathological examination was done using a light microscope. Results reveals that oxidative stress was climaxed at 7th week and was maintained at a constant level while histopathological changes in glomerulus first presented on the 3rd week accompanied by vascular changes. Tubulointerstitial changes were noticed on the 7th week. On the 12th week renal parameters were significantly altered when compared to the animals sacrificed on 3th and 7th week. In conclusion, the sequence in diabetic–induced renal dysfunction begins with changes in vascular and glomerular compartment followed by distortion in tubulointerstitial compartment. An alteration in renal parameters presents lastly and correlates with the histopathological changes. These findings can be adopted in clinical management and treatment of diabetes-induced kidney dysfunction.

Key words: Diabetic nephropathy, kidney, oxidative stress, renal parameters.

Introduction

Diabetes mellitus has been reported as the most common cause of end stage renal disease (Nehel et al. 2018). One of the complications associated with diabetic mellitus is diabetic nephropathy, a disorder characterized accumulation of extracellular matrix in the glomerular and tubulointerstitial compartments of the kidney which may result in renal failure (Nangaku 2004). Hyperglycaemia-induced injury results from

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excessive generation of Reactive Oxygen Specie (ROS) causing a change in intraglomerular dynamics (Singh et al. 2008).

The pathogenesis of DN is mainly explained from genetic susceptibility factor, abnormal glucose metabolism pathway, kidney hemodynamic changes, inflammatory response theory and cytokine theory (Li et al. 2018). Researchers have described cellular events during diabetic nephropathy to include generation of ROS, channeling of glucose intermediates into metabolic pathways resulting in generation of advanced glycation product and the increase in expression of transforming growth factor. The result is hyperglycaemia-induced injury from excessive generation of ROS thereby causing an alteration in intraglomerular hemodynamics (Singh et al. 2008). The single administration of streptozotocin (STZ) produced diabetes, which induced renal oxidative stress, altered the lipid profile, and subsequently produced nephropathy in eight weeks by increasing serum creatinine, blood urea nitrogen, proteinuria, and glomerular damage (Mandeep et al. 2019).

Both vascular, glomerular and tubulointerstitial injuries are involved in pathogenetic mechanisms of diabetic nephropathy. Increase in hyperglycaemia ambience within the kidney tissue will creates a redox environment in the vasculature, nephron and surrounding interstitium causing a dysfunction of virtually all types of kidney cells (Phillips, 2003) and correlates with alteration in renal parameters such as Glomerular Filtration Rate (GFR), proteinuria and decrease in creatinine clearance (Nangaku 2004).

Oxidative stress caused by increased levels of ROS serve as an inducer and amplifier of signaling cellular events that occur in high glucose ambience (Anyanwu and Agbor 2021, Ha et al. 2005, Brownlee 1995), ROS is required in small amounts necessary to maintain cellular homeostasis, but sustained hyperglycaemia in diabetic conditions will cause a dramatic rise in ROS thereby damaging target organs (Lee et al., 2003). During oxidative metabolism in the mitochondria, high concentration of oxygen is reduced to water, while part of oxygen though little is transformed to O_2 (Brand 2010). Hyperglycemia can also cause increased levels of ROS that is capable of resulting in cellular dysfunction and mutations (Nishikawa et al. 2000). Agbor et al. (2020) and Anyanwu and Agbor (2020) have demonstrated and implicated high hyperglycaemic ambience in increased ROS as having a correlation with histopathologic cascade resulting in cellular damage.

The correct sequence of histopathological events that leads to diabetic nephropathy is not well established; therefore this study investigated the changes in both microstructure of kidney tissue and the corresponding alteration in renal parameters following sustained hyperglycaemia in diabetic model.

Materials and Methods

Experiments with animals

Twenty (20) adult male with average weight of 150g were purchased from the department of pharmacology, university of Calabar, Nigeria and used for this research. Animals were handled according to Animal Care and Use in Research, Education and Testing (ACURET) guidelines. The experimental protocol lasted for a period of 14 weeks. The rats were kept in clean cages and divided into four designated A, B, C and D with five rats in each group. The rats were allowed to acclimatize for two weeks in animal house, Department of Anatomy, Faculty of Basic Medical Sciences, University of Calabar, Nigeria and allowed unrestricted access to commercially available chow (livestock feed) and water. Group A served as normal control while groups B, C and D were induced with diabetes and allowed for 3 weeks, 7 weeks and 12 weeks respectively.

Ethical approval and consent

Ethical clearance for this research was obtained from the Ethical Committee, Faculty of Basic Medical Sciences, University of Calabar, Nigeria with Reference number: FBMS/EC/19/056. Animals were handled according to Animal Care and Use in Research, Education and Testing (ACURET) guidelines.

Induction of hyperglycaemia

After fasting for twelve hours, hyperglycaemia was induced by administering streptozotocin (STZ) intra-peritoneally, reconstituted in 0.5M Sodium citrate and administered at a dose of 65mg/kg.bw (Ugochukwu and Babady 2003).

Confirmation of diabetes

Diabetes was confirmed three days after administration of STZ using Accu-Check glucometer (Roche diagnostic, Germany) with blood samples obtained from tails of Wistar rats. Blood glucose levels (mmol/l) were checked weekly to ascertain hyperglycaemic state (Agbor and Anyanwu 2020).

Termination of experiment and collection of samples for analysis

At the end of experimental protocol, the animals were sacrificed by cervical dislocation, at different time based on the number of weeks each group represents. The kidneys were harvested weighed together using an electronic weighing balance (Mettler Instrument AG, Switzerland) and suspended in 40% buffered neutral formaldehyde for further processes with conventional histological techniques. Sections were cut at 5.0 µm, stained in Heamatoxylin and Eosin (H & E) and examined under a light microscope.

Evaluation of oxidative stress markers

Oxidative stress markers were analyzed using blood obtained by cardiac puncture. Samples were transported to the laboratory for biochemical study. Oxidative stress marker kits (Sigma-Aldrich Products, Germany) were used to demonstrate for soperoxide dimutase (SOD), catalase and melondialdehyde (MDA) (Agbor et al. 2021).

Analysis for renal parameters

A 24 hours urine collection was obtained from metabolic cages housing each group of the experimental animals few hours before sacrifice and examined. Urine and serum creatinine was measured using creatinine auto-analyzer (Beckman Instrument, Fullerton CA). Total protein was measured by spectrophotometric assay as modified by Lowry using bicinchoninic reagent (Sharma et al., 2001).

Statistical analysis

Statistical significance of the differences among the groups was determined using one way analysis of variance (ANOVA) with SPSS statistical analysis program version 20.0. $p<0.05$ was considered significant.

Results

Weight of kidney

As shown in Fig. 1, a significantly ($p<0.05$) decreased kidney weight (1.24 ± 0.67 g) was observed at the 7th week when compared to 1.82 ± 0.65g recorded by normal control. However, weight of kidney on the 12th week was further reduced to 1.02 ± 0.34 g and this value was significantly ($p<0.05$) lower when compared to group B (3 weeks of sustained hyperglycaemia)

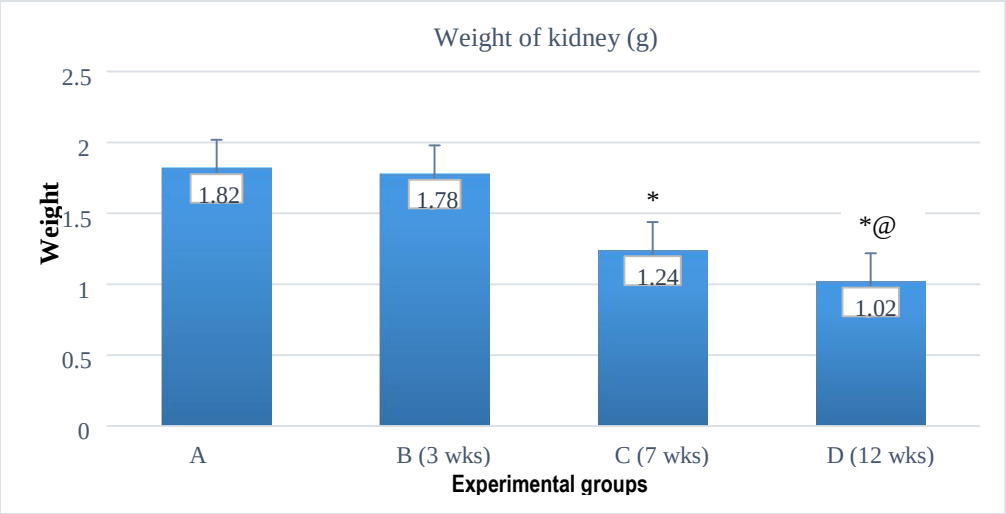

Fig 1: Comparison of weight of kidney in different experimental groups.

Values are expressed in Mean ± SEM. N = 5, *= Values are remarkably decreased when compared to Normal Control at p<0.05. @ = Values are significantly increased when compared to Diabetic Control at p<0.05.

Biochemical analysis

Serum levels of SOD, Catalase and MDH where significantly (p<0.05) higher at 7th week of sustained hyperglycaemia when compared to the normal control. SOD (6.44±0.11 as against 2.50±0.34), Catalase (2.24±0.45 as against 1.04±0.19) and MDH (2.45±0.15 as against 0.16±0.77). There was no significant difference between values of serum oxidative stress markers at the 12th and 7th week of sustained hyperglycaemia (Table 1).

Table 1. Comparison of blood glucose levels and oxidative stress markers in the different experimental groups.

	Experimental groups			
Parameters	A (NC)	B (3 w)	C (7 w)	D (12 w)
Blood glucose (mmol/L)	88.01±0.12	148.32±0.55*	210.44±0.26*@	253.01±0.45*@
SOD (mmol/L)	2.50 ± 0.34	3.91 ± 0.11	6.44 ± 0.11*@	7.01 ± 0.13*@
Catalase (Katf)	1.04 ± 0.19	1.23 ± 0.10	2.24 ± 0.45*	2.29 ± 0.34*@
MDH (µmol/L)	0.61 ± 0.77	0.95 ± 0.21	2.45 ± 0.15*@	2.84 ± 3341*@

NC = Normal Control, SOD = Superoxide dismutase, MDH = Melondialdehyde. Values are expressed in Mean ±SEM. N = 5, * = Values are significantly increased when compared to Normal Control at p<0.05. @ = Values are significantly increased when compared to Diabetic Control at p<0.05.

Histo-pathological examination

Figure 2a shows kidney section in non-diabetic animals (Normal control) having prominent renal tubules and glomeruli with an intact bowman space. Animals left under hyperglycaemic condition for 3 weeks (Group B) shows prominent renal tubules and glomeruli. There was mesangial cell proliferation and thickening of glomerular basement membrane. The intervening stroma is scanty and consists of sparse interstitial cells. The glomeruli had intact bowman space and a cellular mesangium (Fig. 2b). As shown in figure 2c section of the kidney in group C places on 7 weeks of sustained hyperglycaemia had scanty intervening stroma with mild inflammatory infiltrates. The glomeruli are atrophic with a large Bowman's space. At 12th week (Group D), histological section of kidney revealed diffused expansion and proliferation of messangium, thickening of glomerular basement membrane, renal tubular hypertrophy, scanty and sparse interstitial cells with inflammatory infiltrates, distorted Bowman's space and extensive haemorrhage within the mesangial matrix (Fig. 2d).

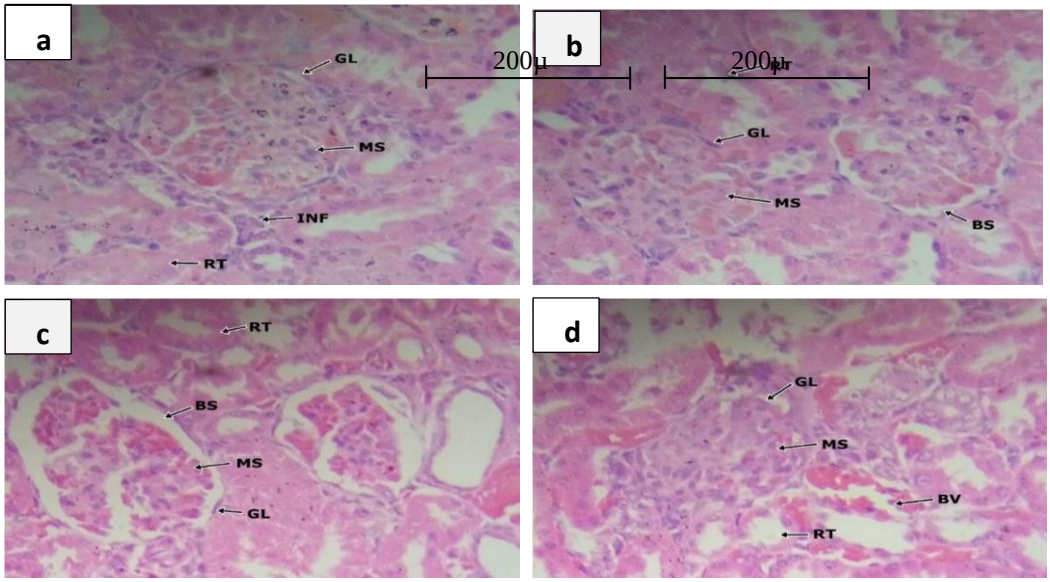

Fig. 2 (a-d): Histopathological observation of kidney.

GL = Glomerulus, MS = Mesangium RT = Renal tubule

BS = Bowman's space, BV = Blood vessel

200µ 200µ

Renal parameters

Analysis of renal parameters in Table 2 reveals that all values of serum creatinine, creatinine clearance, and proteinuria at 12 weeks of sustained hyperglycaemia (group D) put at 3.21±0.08 mg/dl, 2.77±0.19 L/kg/D and 334.61±0.25 mg/kg/D respectively were significantly higher when compared to the normal control (group A), groups C and D.

Table 2. Comparison of renal parameters in the different experimental groups.

Renal parameters	*Experimental groups*			
	A	B	C	D
	NC	3 w	7 w	12 w
Serum creatinine (mg/dl)	0.71±0.90	0.98±0.64	1.15±0.25	3.21±0.08*@
Creatinine clearance (L/kg/Day)	6.01±0.11	5.92±0.34	5.71±0.56	2.77±0.19*@
Proteinuria (mg/kg/Day)	104.18±0.11	111.19±0.11	120.32±0.83	334.61±0.23*@

NC = Normal control, W = Weeks.

Values are expressed in Mean ± SEM. N = 5. * = Values are significantly decreased (creatinine clearance) and increased (serum creatinine and proyeinuria) when compared to Normal control at $p<0.05$. @ = Values are significantly increased (serum creatinine and proyeinuria) when compared to diabetic control at $p<0.05$.

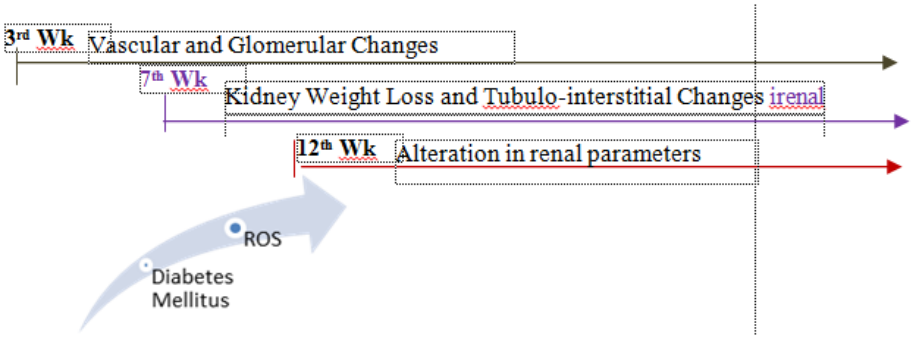

Fig. 3: An illustration of the sequence of histopathological changes occasioned by sustained hyperglycaemic ambience in an experimental diabetic nephropathy.

Discussion

It has been revealed from this research (Table 1) that increase in hyperglycaemic levels is directly proportional to levels of oxidative stress generation and in line with this observation, extensive experimental evidence suggests that hyperglycaemic changes can result in excessive generation of ROS capable of causing changes in cellular elements in the kidney (Lenzi et al. 1993, Aitken and Krausz 2001). This may occur by way of modifying all bases, free sites deletion, shift in frame, DNA cross-link and chromosomal rearrangement thereby causing cellular damage. Unfortunately, all cellular elements in the kidney respond to hyperglycaemic ambience by the activation of similar intracellular signaling pathway though with little variation depending on specific molecules expressed by various cell types. This finds support in Yashpal et al. (2011), who demonstrated that high glucose ambience induces intracellular events which include increased flux of polyols and hexosamines; generation of advanced glycation end products (AGEs) and reactive oxygen specie (ROS).

This study has revealed a significant weight loss in the kidney of experimental diabetic animals sacrificed on 7th and 12th week when compared to the normal control, even though diabetic animals sacrificed on the 3rd week did not show any remarkable differences in terms of kidney weight when compared to the normal control (Fig. 1). It can therefore be suggested that weight loss in kidney corresponded to the duration of hyperglycaemia within the micro environment of the kidney which may have caused significant reduction in weight of organ. Weight of an organ depends, to a very large extent, on the mass of various cells. According to Wolf (2004), hyperglycaemic injury affects all cell types in the kidney including mesangial, vascular endothelia interstitial and fibroblast cells at varying degrees and this may have given rise to weight loss. Researchers have revealed that all cell types in the kidney, vascular endothelia, interstitial fibroblast, glomerular podocytes, tubular epithelia, mesangial and endothelial cells are affected by hyperglycaemic injury at varying levels and as a consequence, the progressive reduction in weight of kidney.

Fioretto and Mauer (2007) reported that glomerular, tubulointerstitial and vascular changes constitutes pathogenicity of diabetic nephropathy. This study clearly ascribes the initial stages of diabetic nephropathy to glomerular and vascular alterations. Glomerular changes first appeared on the 3rd week and were shown in thickening of glomerular basement membrane with very mild inflammatory infiltrates which implies that vascular and glomerular alterations occurred. Changes in the tubulointerstitial compartment included inflammatory infiltrate and tubular hypertrophy was observed at the 7th week accompanied by further degenerative changes in the glomerulus like diffused expansion of the mesangium with various degrees of hemorrhage within the mesangial matrix. At 12th week, hyperglycaemic-inuced injury caused much more deleterious effects within the glomerular, vascular and tubulointerstitial compartments accompanied by extensive hemorrhage.

Findings from this research also reveal that all renal parameters were not significantly different at the 3rd and 7th weeks of sustained hyperglycaemia when compared to normal control. However significant changes recorded on the 12th week was indicative of kidney dysfunction. It is therefore reasonable to suggest that changes in creatinine clearance, serum creatinine levels and proteinuria concentration as observed in this study does not only confirm a correlation between renal parameters and hyperglycaemia-induced kidney injury but also shows that the appearance of these altered indicators is in the later stages of histopathologcal changes and could serve as a clinical evidence.

Conclusion

This research has established the sequence of degenerative changes as a consequence of increased oxidative stress generation associated with hyperglycaemia-induced kidney injury in diabetic animal model. Vascular and glomerular changes were first observed, followed by tubulointerstitial changes and then alterations in renal parameters. Understanding the sequence of this degenerative process can provide knowledge about the pathogenesis of diabetic nephropathy which may be found useful in clinical management and treatment of diabetic induced kidney dysfunction and related complications.

Competing interests

This research is not by any means linked to any funding body and so there was a complete absence of competing interest.

Authors' contributions

All authors performed all relevant tasks involved in this study.

Acknowledgments

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